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The Effect of Temperature and Other Factors on Selective Microvascular Damage Caused by Pulsed Dye Laser

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Brief pulses of 577-nm radiation have recently been shown to selectively damage superficial cutaneous blood vessels, resulting clinically in purpura. There was a sharp threshold of exposure dose necessary for causing purpura in any given subject, which correlated with histologic evidence of extravasation and specific vascular injury. As a means of studying mechanisms for such damage, heat, cold, pressure, suction, UV radiation, and intradermal epinephrine were used to alter human cutaneous microvasculature prior to and during 577-nm pulsed dye laser exposures. When compared with control sites, only cooling of the skin significantly affected the exposure dose needed to cause purpura. The magnitude of this effect is quantitatively most consistent with intravascular microvaporization as the cause of vessel rupture and hence purpura.

In Caucasian skin, superficial cutaneous blood vessels are the major sites absorbing 577-nm radiation (green-yellow visible light). When absorbed, this wavelength therefore generates heat specifically within superficial blood vessels, and if delivered in a brief, intense pulse can cause selective damage to cutaneous microvessels. Accordingly, it has recently been shown that single, 3×10^{-7} s (300 ns)-duration pulses of 577-nm radiation from a pulsed tunable dye laser selectively damage Caucasian cutaneous microvessels in vivo at exposure doses of 2.0 J/cm^2 or more [1,2]. Histologically, rupture and hemorrhage of the superficial vascular plexus were noted with associated endothelial and vessel wall necrosis. All vessels to a depth of 0.5 mm were affected. A pattern of acute vasculitis evolved with fibrin, polymorphonuclear leukocytes, and karyorrhexis in the necrotic venular walls. Few or no alterations were apparent at the light microscopic level in dermal fibroblasts, collagen, elastic tissue, or in the overlying epidermis, despite significant vascular damage. The same exposure doses that caused histologically specific effects on blood vessels also caused purpura in the exposed skin. This purpura was an all-or-none response occurring within minutes at most and usually immediately after exposure.

Although it is clear that purpura results from extravasation of blood, it is not clear which of several possible damage mechanisms may be causing this superficial hemorrhage. Possibilities include thermal denaturation, vaporization, and shock wave damage [3–5]. At temperatures less than 100°C, protein denaturation, membrane disintegration, and rapid endothelial and pericyte cell necrosis may weaken the vessel wall enough to cause hemorrhage and purpura. Alternatively, or in addition, intravascular vaporization of erythrocytes or plasma may occur,

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Abbreviations:
MED: minimal erythema dose

and the sudden expansion caused when steam is generated may literally explode the vessels. Another possibility is that shock waves caused by rapid spatial and temporal thermal transients, in the absence of vaporization, might propagate and disrupt weak points in vessel walls. With this particular laser the estimated rate of heating of microvascular blood is on the order of 10^8 degrees C per s [2]. Some of these possible mechanisms require that a certain temperature be reached, i.e., 100°C or more for vaporization, and approximately 75°C or more for protein denaturation. Damage by these mechanisms should therefore depend in part upon the ambient temperature of the skin, with cooler skin requiring more energy input to reach the damaging temperature required. Other mechanisms, such as thermally induced shock wave damage, depend mainly upon localized temperature differences and transients, rather than absolute temperatures, and might be much less affected by differences in the initial skin temperature. In addition, it may be argued that states of vasodilatation or blood flow could effect the integrity, elasticity, or strength of microvessels, as well as altering the amount of blood present, hence optical absorption and/or thermal diffusion capacity. In order to differentiate among some of the possible mechanisms involved in causing purpura, we decided to alter skin temperature, hemodynamics, and vasodilatation using heat, cold, pressure, suction, UV radiation, and vasoactive pharmacologic agents, prior to and including the time of 577-nm dye laser exposures. The effect of these manipulations on the threshold exposure dose for causing purpura after a single laser exposure was determined and analyzed.

MATERIALS AND METHODS

A flashlamp-pumped organic dye laser (Candela Corp., model #SLL-1100) was used, producing 3×10^{-7} s (300 ns) pulsewidth, typically 500 mJ pulses using Excitron Inc. rhodamine 575 dye 10^{-4} M in methanol. The laser's output was tuned to 577 nm and focused with a planoconvex lens into a single 1 mm-diameter 2 m-long, polished, flexible quartz step-index fiber (Math Associates Q1000). A seated planoconvex field lens assembly was used near the output tip of the fiber to produce a 3 mm-diameter uniform exposure field on the skin. The irradiation striking the skin was a uniform magnified real image of the fiber tip. A valve communicating with the field lens assembly permitted either air suction or pressure to be applied to the skin during exposure. Dosimetry was calculated by measurement of total beam energy at the level of the skin with a Scientek model 362 laser energy meter. Pulses were always repeatable to within 5%.

Six paid, informed, and consenting volunteer subjects received a series of single-pulse exposures on the upper buttocks with a range of exposure doses (incident energy densities) in 0.25 J/cm² increments from 0.5 J/cm² to 2 J/cm². Exposures, done in adjacent areas, were given to skin at ambient temperature (~33°C), heated (~42°C), cooled (~10°C), made erythematous by 100 mm Hg suction applied for 10 s, blanched by 250 mm Hg pressure applied for 10 s, blanched by prior intradermal injection of 1 cc of epinephrine 1:100,000, and in 3 subjects, to a patch made erythematous by UVB (290–320 nm) radiation given the previous day by exposure to FS40 sunlamps. The temperature changes were induced by applying 2-liter pyrex beakers of either 50°C heated water or 4°C ice water for 5–10 min prior to exposure. Skin surface temperature adjacent to the exposure site was monitored with a small thermocouple and Digitec Ht-5820 digital thermometer immediately prior to each exposure. The threshold for purpura was defined as the lowest incident energy density (exposure dose) which resulted in purpura observed within 30 min following exposure. As an additional control, 5 exposures, each at one-half the threshold for purpura were given to a single site at 1-s intervals in all subjects. Thus, an exposure dose greater than twice that causing purpura when given in a single exposure was given in a manner not expected to induce purpura. Exposures to ambient temperatures and cooled skin (~10°C) were then repeated in 8 additional subjects over a range of 0.9 J/cm² to 1.8 J/cm² in 0.1 J/cm² increments to more closely examine the effect of temperature. In 4 of these subjects the skin was also similarly exposed at 42°C and 20°C.

RESULTS

Purpura was induced within the first 2 min after exposure. Most of the vasoactive manipulations had little if any effect on the energy required for purpura formation as shown in Table

TABLE I. *Thresholds for production of purpura in J/cm²*

Subject no.	Normal (~33°C)[a]	Heat (~42°C)[a]	Cold (~10°C)[a]	Suction	Pressure	Epinephrine	UV erythema
1	1.5	1.5	1.75	1.25	1.5	1.5	1.5
2	1.25	1.5	1.5	1.25	1.0	1.5	1.25
3	2.0	1.75	2.0	2.0	2.0	2.0	
4	1.25	1.0	1.5	1.0	1.25	1.5	1.5
5	.75	.75	1.0	.75	1.0	1.0	
6	1.0	.75	1.25	1.0	1.25	1.0	
Ave ± SD	1.29 ± 0.43	1.20 ± 0.43	1.50 ± 0.35	1.21 ± 0.43	1.33 ± 0.38	1.42 ± 0.38	1.41 ± 0.14
Significance (vs 33°C data) by paired *t*-test		NSD[b]	$p < .01$	NSD	NSD	NSD	NSD

[a] Skin surface temperature immediately prior to laser exposure.
[b] NSD = no significant difference by paired *t*-test.

TABLE II. *Purpura threshold in J/cm²*

Subject no.	10°C (cooled)	20°C (cooled)	33°C (normocaloric)	42°C (max, heat)
1	1.6	N/D	1.2	N/D
2	1.7	N/D	1.5	1.5
3	1.6	N/D	1.4	1.4
4	1.7	N/D	1.3	1.4
5	1.4	1.3	1.1	N/D
6	1.7	1.6	1.5	N/D
7	1.6	1.4	1.0	N/D
8	1.5	1.3	1.2	1.3
Ave ± SD	1.60 ± 0.11	1.40 ± .14	1.27 ± .18	1.40 ± .08
Significance (vs 33°C) by paired *t*-test	$p < 0.001$	NSD		NSD

I. The threshold dose for purpura observed after heating, suction, pressure, intradermal epinephrine, or UV erythema were not significantly different from control values. Only with cooling was there a consistent change, with 5 of the 6 subjects having a greater threshold energy. When further investigated in 8 additional subjects using 0.1 J/cm^2 increments, a significant increase in the purpura threshold of approximately 0.33 J/cm^2 was noted when the skin was cooled to 10°C (Table II). Cooling to only 20°C resulted in an apparent but statistically insignificant increase in threshold by 0.13 J/cm^2, less than that seen with cooling to 10°C. Heating resulted in no significant changes in purpura threshold. In no subject did the 5 repeated exposures to the same exposure site of ½ the single-pulse-purpura threshold dose at 1-s intervals result in a purpura response.

DISCUSSION

No difference in threshold dose was noted under conditions of suction, pressure, epinephrine blanch, or UVB erythema, all of which change the state of dilatation and blood flow in microvessels, and the quantity of superficial blood present in the skin. These data argue strongly for the importance of damage mechanisms which are relatively independent of the state of vasodilatation or blood flow.

Only cooling of the skin caused a consistent increase in the threshold for purpura. This suggests, as mentioned, that purpura is caused by the attainment of some relatively fixed peak vessel temperature. Prior cooling may increase the purpura threshold because more energy is required to reach that temperature at which hemorrhage occurs if one starts from a lower baseline temperature. If the purpura were caused by shock waves associated with rapid thermal expansion other than microvaporization, there would be little or no dependence of the purpura threshold on the initial skin temperature, because the thermal expansion coefficient of water is relatively constant between 4°C and 100°C. Thus, vaporization or denaturation damage mechanisms must be considered, both of which meet the condition that purpura is caused by the attainment of some critical peak temperature causing vessel rupture.

A temperature of approximately 75°C or more will cause denaturation of proteins, DNA, and collagen; many proteins, enzymes, and membranes are damaged at considerably lower temperatures. If vaporization is the major damage mechanism leading to purpura, a temperature of over 100°C must be involved. The putative peak temperature causing purpura can be estimated from the data at hand if several simple assumptions are made. Firstly, we assume that no significant cooling of blood vessels occurs *during* the exposure pulse. This is a reasonable assumption because the estimated time for even the smallest vessels present to cool significantly is on the order of 10^{-4} s [2], about 300 times the 3×10^{-7} s laser pulse duration. Because there is no significant vessel cooling during exposure, the temperature increase within any given vessel during the laser exposure is directly proportional to the absorbed energy per unit volume in that vessel. This absorbed energy, in turn, is directly proportional to the incident energy density or exposure dose. This proportionality is, of course, true only for the liquid state; after reaching approximately 100°C, further energy input is invested primarily in the heat of vaporization of water. Past this vaporization point, the volume of steam generated will be roughly proportional to the absorbed energy and, hence, incident energy density.

These concepts are shown diagrammatically in Fig 1, which assumes simple linearity between incident energy density and heat input to a vessel during exposure. The difference of .325 ± 0.14 J/cm^2 incident energy density noted between purpuric thresholds at 33°C and 10°C is statistically significant ($p < 0.001$ by paired t-test) and corresponds to a temperature rise of $\frac{(33-10)°C}{0.325\ J/cm^2}$ or 71°C per incident J/cm^2. As an estimate in these

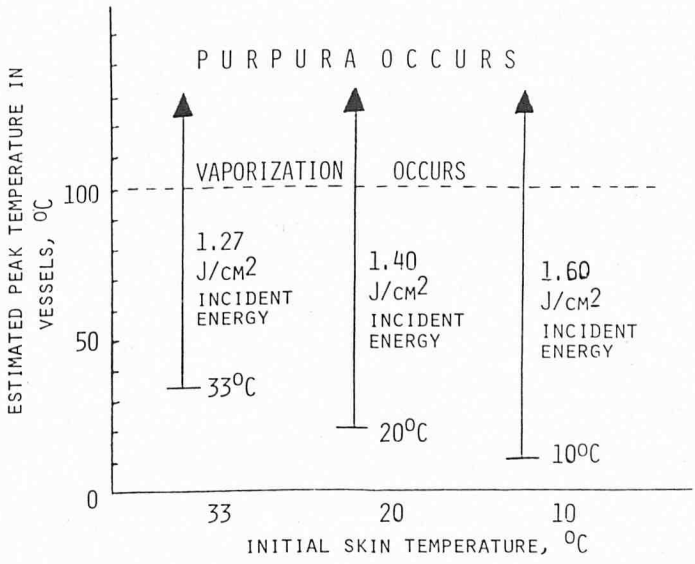

FIG 1. Simple model depicting approximate linearity between the maximum vessel temperature achieved and the incident laser energy density (577 nm, 300-ns pulse). The data are most consistent with microvaporization of blood within cutaneous vessels as the major damage mechanism leading to purpura formation.

subjects an equivalent peak temperature in the vessels involved of about (1.27 J/cm^2 × 71°C/J/cm^2) + 33°C, or 123°C, was needed to cause purpura.

Thus, 123°C is the estimated peak temperature in the absorbing vessel at the end of the laser pulse. Because this temperature is well over 100°C, vaporization of a fraction of the blood or erythrocytes within the vessel should occur, making this a likely mechanism for damage.

The explosive volume expansion and subsequent sudden collapse that must result from localized intravascular vaporizations may therefore be the major factor in producing vessel rupture and purpura. More precisely, this peak temperature would exist only during a brief instant, as pressurized, superheated steam is generated. Heat would then be expended in vaporizing tissue water and the temperature would rapidly decrease to 100°C as the steam expanded, seeking pressure equilibration with the tissues. As heat further dissipates and the intraluminal blood temperature drops, the gaseous spaces suddenly created by steam generation would then largely collapse by rapid condensation.

If cooling the skin causes an increase, heating the skin should result in a decrease in the purpura threshold, other factors being equal. The thresholds for purpura obtained with a skin surface temperature of 42°C are not, however, significantly different from those at 33°C in the 4 subjects examined at this higher temperature. Heating the skin surface to 42°C represents the warmest, easily tolerated surface temperature, but even discomfort and erythema occur at this temperature. It may be that an increase of only 9°C was too small to result in an observable change in the purpura threshold within this experimental design. If intravascular microvaporization is the damage mechanism responsible for vessel rupture, then it is not surprising that the other manipulations that altered the state of vasodilatation did not change the purpura threshold. Regardless of the state of vasodilatation, the red blood cells nearest the vessel wall closest to the incident laser pulse would vaporize, causing vessel rupture.

In summary, this study indicates that the state of cutaneous vasodilatation, manipulated by vasoactive treatments or agents, has little or no effect upon the specific microvascular damage induced by pulsed dye laser at 577 nm, as evidenced by little or no change in the exposure dose needed to cause purpura in Caucasian skin. However, temperature cooling of the skin

increases the exposure dose needed to cause purpura. The temperature dependence of 300 ns, 577-nm pulsed laser-induced purpura is most consistent with intravascular vaporization as a damage mechanism causing vessel rupture and the resultant purpura.

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The Effects of High-Dose UV Exposure on Murine Langerhans Cell Function at Exposed and Unexposed Sites as Assessed Using In Vivo and In Vitro Assays

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Exposure of mice to a single large dose of UV radiation leads to a systemic inability of these mice to develop effective contact hypersensitivity (CS) responses to epicutaneously applied dinitrofluorobenzene (DNFB). Although this effect requires time to develop when unirradiated skin sites are used for CS sensitization, it is observed immediately at the site of UV exposure. Unirradiated skin sites on mice exposed to a single large dose of UV radiation 3 days previously were found to contain histochemically detectable ATPase$^+$ cells with normal morphology and in normal densities, and yet CS responses were not induced to DNFB applied to these sites. Epidermal cells (EC) obtained from these skin sites were found to be capable of providing accessory cell (AC) function in in vitro T-cell proliferation assays that was qualitatively similar to EC obtained from unirradiated mice, thus indicating that exposure of mice to a single large dose of UV radiation does not induce a systemic AC dysfunction. Indeed, increased levels of AC activity were obtained in EC prepared from the UV-irradiated skin sites on the third day following UV exposure. This latter effect may be due to an influx of inflammatory cells into the irradiated site in response to the tissue damage caused by the UV radiation. We hypothesize that the inflammatory response induced by the cytodestructive effects of the UV treatment may play a central role in the generation of the systemic suppression of induction of CS responses, perhaps through the induction of acute-phase proteins.

Investigations in recent years indicate that the skin is a complex and vitally important organ [1]. Langerhans cells (LC) have been implicated as playing a crucial role as antigen-processing cells (APC) in the induction of positive immune responses to antigens introduced through the skin [2]. Toews et al have shown that relatively low doses of UV radiation are capable of inactivating epidermal LC as assessed by both the density of histochemically detectable ATPase$^+$ cells and the ability to induce positive contact sensitivity (CS) responses to reactive chemicals applied to the UV-exposed skin [3]. Under these conditions the UV-mediated inability to induce CS responses to dinitrofluorobenzene (DNFB) was found to be a localized phenomenon (i.e., application of DNFB to an unexposed skin site in a UV-irradiated host generated CS responses that were indistinguishable in magnitude from the positive control). These observations were confirmed and extended by Lynch et al, who showed that exposure of murine skin to the moderate levels of UV radiation commonly used to induce both tumor susceptibility and overt skin tumors also results in a milieu in which applied contact sensitizers do not effectively sensitize the animals to subsequent antigenic challenge [4]. In addition, it was found that the effects of UV exposure were

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Abbreviations:
AC: accessory cell(s)
APC: antigen-presenting cell(s)
Con-A: concanavalin A
CS: contact sensitivity
DNFB: dinitrofluorobenzene
EC: epidermal cell(s)
ETAF: epidermal thymocyte-activating factor
IL-1: interleukin 1
LC: Langerhans cell(s)